AD-A189 694 ANALYTICAL PROCEDURES FOR EXTRACTABLE ORGANOTINS IN SOFT TISSUES OF MARINE ORGANISMS(U) NAVAL OCEAN SYSTEMS CENTER SAN DIEGO CA K J MEYERS-SCHULTE ET AL. SEP 87 NOSC/TR-1190 1/1

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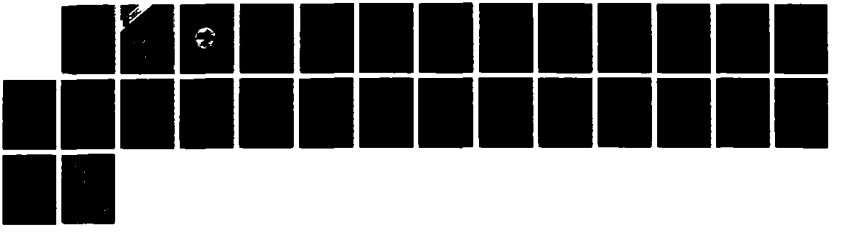

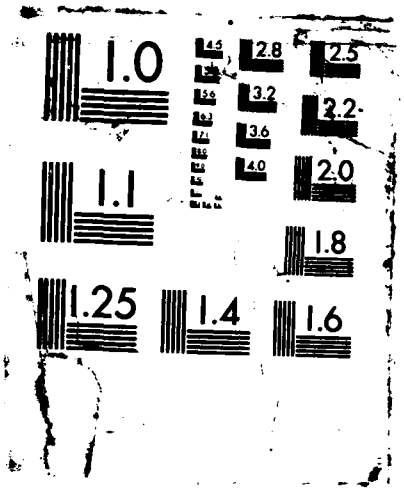
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Technical Report 1198
September 1987

Analytical Procedures for Extractable Organotins in Soft Tissues of Marine Organisms

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ADMINISTRATIVE INFORMATION

The work described herein was performed by the Marine Environment Branch, Code 522, Naval Ocean Systems [illegible] for the David W. Taylor Naval Ship Research and Development C[illegible] and Development Program, and for the Office of Naval Re[illegible]

Released by
P.F. Seligman, Head
Marine Environment [illegible]nch

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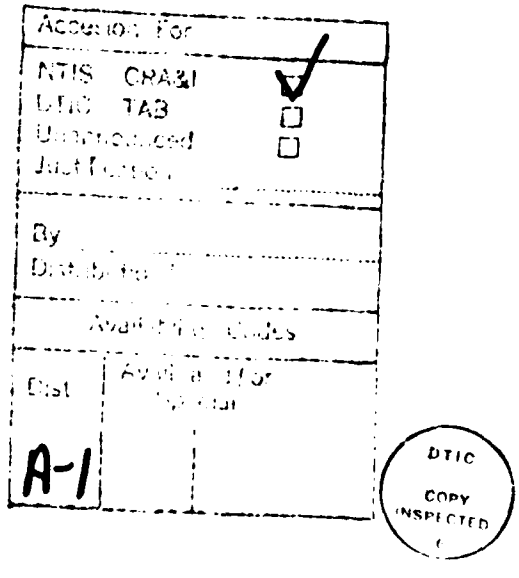


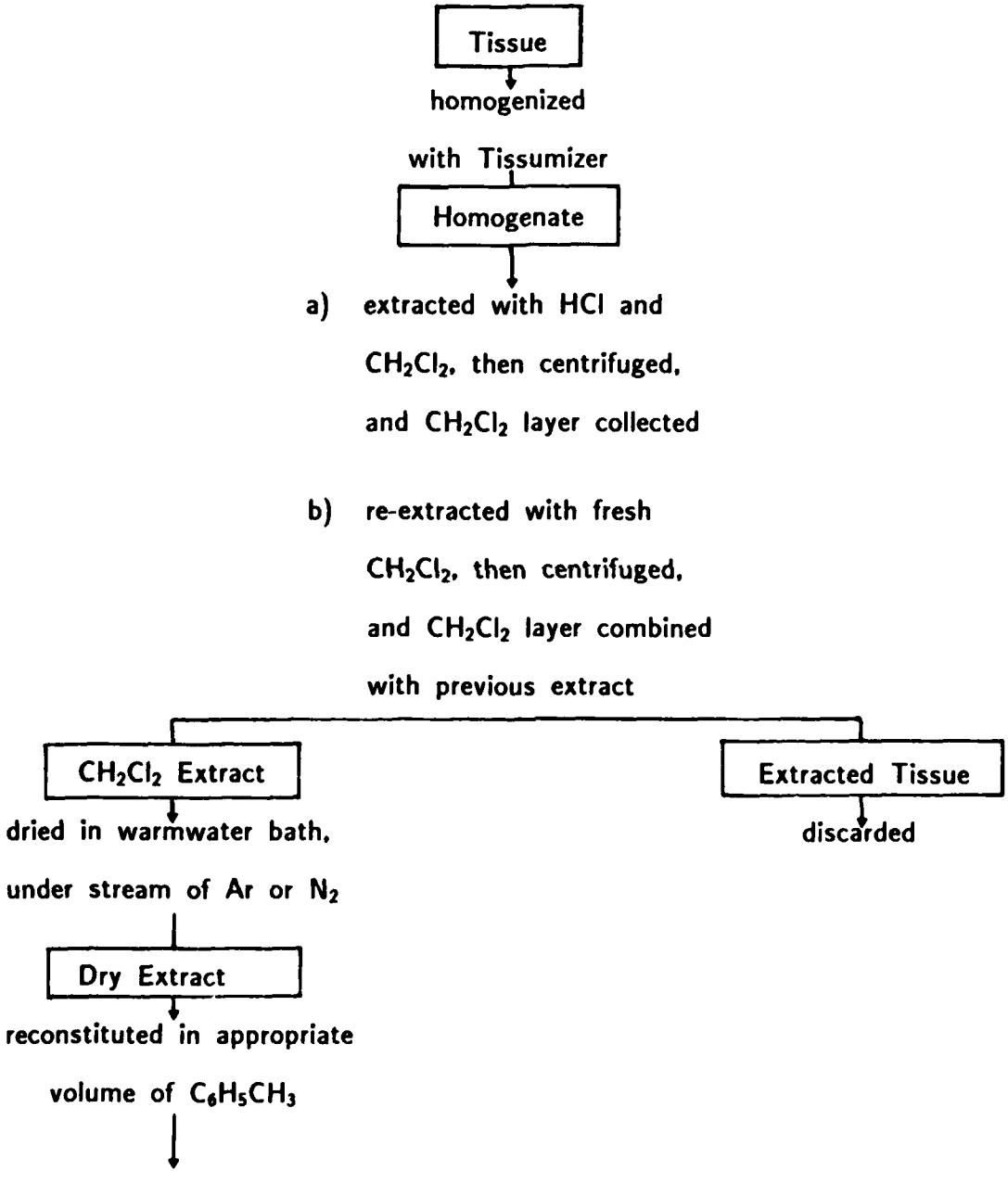
1. INTRODUCTION

There has been increasing national and international concern about the effects of tributyltins on the marine environment. Tributyltins (TBT) are introduced into the waterways predominantly from their use as antifouling agents in marine paints. These coatings, which release the biocide into the water column, are extensively used on commercial and recreational vessels and are proposed for use on naval ships in the near future.

Due to the toxic nature of TBT, there is an extreme need to assess its impact and fate in relation to non-target organisms. To evaluate the interactive roles of TBT and marine organisms, it is necessary to effectively isolate, identify, and quantify TBT and, at times, its alleged degradation products, di- and monobutyltins (DBT and MBT). The analytical procedures presented here are those currently used at the Ocean Sciences Laboratory, Naval Ocean Systems Center, San Diego, to quantify either (a) extractable TBT only, or (b) both TBT and DBT in various marine organisms. Because it is doubtful that any one method will be effective in all situations, development (i.e., improvement) of the analytical procedures is an ongoing process. For this reason, it is stressed that the procedures presented here are (at the time of this writing) most effective for the samples encountered in this laboratory. The thorough (almost cookbook style) presentation is meant to inform the reader of the complete methods currently in use at NOSC and is by no means intended to be an analytical code or rule.

2. OUTLINE AND SUMMARY OF ANALYTICAL PROCEDURES FOR TISSUES

In general, analytical procedures for butyltins in marine organisms follow the outline below.



3. METHODS OF TISSUE PREPARATION

3.1 TISSUE EXTRACTION, DAY 1

Equipment

Glassware and Plasticware (per sample)

101- X 38-mm round-bottom polycarbonate centrifuge tube with screw cap (NALGENE)*
200- X 25-mm culture tube with Teflon-lined screwcap (KIMAX)
127- X 28-mm round-bottom SORVALL centrifuge tube (PYREX), two per sample
125-ml Erlenmeyer flask

Chemical Solvents and Reagents

Water for cleanup, ~300 ml for one to three samples
Ethanol (C_2H_5OH) for cleanup, ~150 ml for one to three samples
6 M hydrochloric acid (concentrated ULTREX HCl, diluted with distilled water), equal weight as sample
Methylene chloride (CH_2Cl_2), 50 ml per sample

Other Materials

Dissecting tray*
Shucking knife*
Vernier caliper*
Kimwipes
Lab marker
400-ml plastic disposable beaker, one per two samples
Rack for 200- X 25-mm culture tubes and 127- X 28-mm round-bottom centrifuge tubes
500-ml wash bottle for ethanol
Tissumizer (TEKMAR)
Top-loading balance (accurate to 0.01 g)
Beaker for taring, ~400 ml
400-ml plastic disposable beaker for water wash
~4- X 140-cm (100-ml) glass graduated cylinder for ethanol wash
Teflon-coated spatula
100-ml glass graduated cylinder (in addition to the one listed above)
20-ml glass graduated cylinder
1-in.-wide Teflon tape, ~4-in. length per sample
Parafilm sheeting, four squares per sample
Polyethylene gloves
Rototorque (COLE-PARMER)
Compressed argon or nitrogen gas
Mini-vap (SUPELCO), one outlet per sample

*Necessary if animals have not been dissected.

~5- X 30- X 19-cm tray, one per six samples
5- to 10-ml pipettor
Disposable tips for 5- to 10-ml pipettor, one per sample

Procedure

(1) With a lab marker, label 200- X 25-mm culture tubes (one per sample) and 127- X 28-mm round-bottom SORVALL centrifuge tubes (two per sample), and place them in a rack.

(2) Cut 1- to 1.5-in. lengths of 1-in.-wide Teflon tape (four per sample), and keep them clean on a Kimwipe.

(3) Cut squares of Parafilm sheeting (four per sample); keep them clean on a Kimwipe.

For animals not previously shucked into 101- X 38-mm polycarbonate centrifuge tubes, continue with step 4; otherwise proceed with step 14.

(4) Thaw frozen specimens in their storage bags at room temperature.

(5) Label 101- X 38-mm polycarbonate centrifuge tubes (three replicate tubes per sample bag) with labeling tape.

(6) Pool three groups of five to ten animals each (of 45-60 mm length if mussels) and place them on a dissecting tray.

(7) Using a vernier caliper, measure and record the length of each animal in each set to an accuracy of 0.01 mm (optional).

(8) Open the shell with a shucking knife and record the reproductive stage of each animal (optional).

(9) Blot the interior with a fresh Kimwipe.

(10) (Discard the byssal threads of mussels.) Cut away all tissue from the inner shells.

(11) Carefully transfer the tissue of all animals in each set into their correctly labeled polycarbonate centrifuge tubes and cap the tubes.

(12) With water and then ethanol, thoroughly rinse the tray and knife, letting them air-dry before being used for the next sample batch.

(13) Clean the Tissumizer by immersing the rod in a 400-ml beaker of water and switching the controller "ON" for 5 s. Wipe the rod with a Kimwipe, then immerse the rod into a 4- X 140-cm (100-ml) graduated cylinder of ethanol, and again switch the controller on for ~5 s. Be sure to keep at least the bottom inch of the Tissumizer rod submerged while it is running. Let the Tissumizer air-dry before using it for tissue homogenization.

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REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) NOSC TR-1198	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION Naval Ocean Systems Center 6b OFFICE SYMBOL (if applicable) Code 522	7a NAME OF MONITORING ORGANIZATION
6c ADDRESS (City, State and ZIP Code) San Diego, CA 92152-5000	7b ADDRESS (City, State and ZIP Code)
8a NAME OF FUNDING / SPONSORING ORGANIZATION David W. Taylor Naval Ship Research and Development Center 8b OFFICE SYMBOL (if applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State and ZIP Code) Annapolis, MD 21402	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 63724N; PROJECT NO: Z0838; TASK NO: 522-ME38; AGENCY ACCESSION NO: DN888 749

11 TITLE (Include Security Classification)
Analytical Procedures for Extractable Organotins in Soft Tissues of Marine Organisms

12 PERSONAL AUTHOR(S)
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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM Feb 1986 TO Jan 1987	September 1987	

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB GROUP	Tributyltin; Dibutyltin; Biocides; Marine pollution; Graphite furnace atomic absorption; Gas chromatography

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

A method for isolating extractable tributyltin and dibutyltin from soft tissues of marine organisms is presented. Analyses by graphite furnace atomic absorption spectrophotometry and by gas chromatography with both flame ionization detection and mass spectrometry are described.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED / UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Carol A. Dooley	22b TELEPHONE (Include Area Code) (619) 225-6850
	22c OFFICE SYMBOL Code 522

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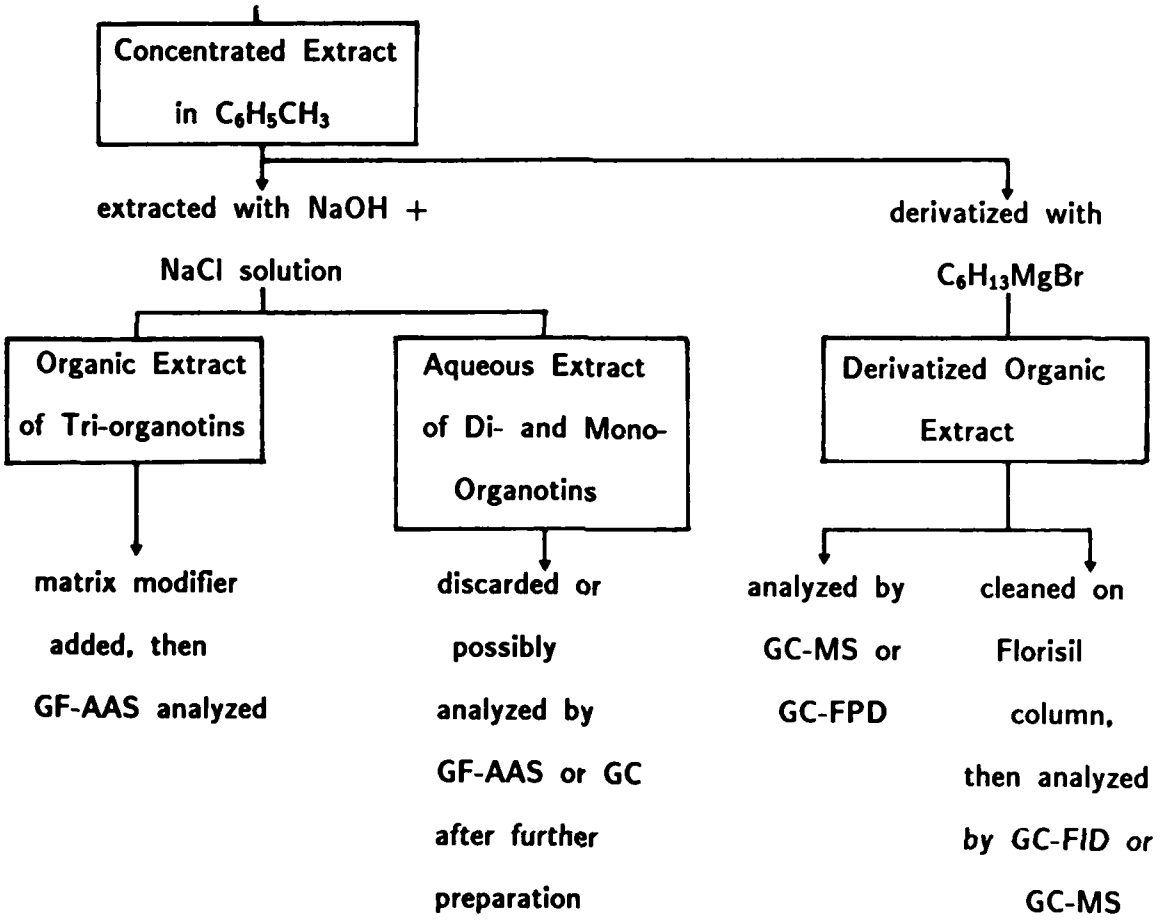
Concentrated Extract in $C_6H_5CH_3$
extracted with NaOH + NaCl solution
derivatized with $C_6H_{13}MgBr$
Organic Extract of Tri-organotins
Aqueous Extract of Di- and Mono-Organotins
Derivatized Organic Extract
matrix modifier added, then GF-AAS analyzed
discarded or possibly analyzed by GF-AAS or GC after further preparation
analyzed by GC-MS or GC-FPD
cleaned on Florisil column, then analyzed by GC-FID or GC-MS

The outline of analytical procedures can be summarized as follows. Thaw the frozen sample, (and if not previously shucked, remove tissue from the shell or carapace, pouring off excess fluid), then grind the tissue. Weigh homogenate (5-17 g), add 6 M aqueous hydrochloric acid (HCl), and extract two times with methylene chloride (CH_2Cl_2). Dry the combined extracts in a warmwater bath under a stream of argon (Ar) or nitrogen (N_2).

Reconstitute the residue in an appropriate amount of toluene ($C_6H_5CH_3$). This concentrated extract can be prepared for either graphite furnace atomic absorption spectrophotometry (GF-AAS), analyzing for tributyltin only, or gas chromatography (GC), analyzing for speciated butyltins.

For GF-AAS analysis, mix the extract with a solution of ~5 M NaCl in 3-percent aqueous NaOH. Discard the aqueous layer or save it for possible further analyses of your choice. To aliquots of the toluene ($C_6H_5CH_3$) layer, make standard additions by adding appropriate volumes of a freshly prepared tributyltin chloride standard, then dilute the solutions with an ammonium dichromate [$(NH_4)_2Cr_2O_7$] and isopropyl alcohol [$(CH_3)_2CHOH$] matrix modifier, and analyze the sample by GF-AAS.

For GC analysis, add an internal standard to the toluene concentrate and derivatize with hexylmagnesium bromide ($C_6H_{13}MgBr$). This hexylated concentrate can be analyzed directly by GC-mass spectrometry (GC-MS) or be cleaned by passage through a Florisil column before either GC-flame ionization detection (GC-FID) analysis or GC-flame photometric detection (GC-FPD) analysis.

(14) (For tissue samples that were frozen in the polycarbonate centrifuge tubes, thaw the samples at room temperature before proceeding.) For each polycarbonate tube of tissue, grind the sample until it appears homogeneously smooth (should take from 1 to a few minutes). Try not to let the sample heat up during this process.

(15) Wipe the Tissumizer with a Kimwipe and clean the rod as in step 10. Replace dirty water and ethanol with clean batches, at least after each three uses.

(16) Set the 400-ml taring beaker on the top-loading balance. Into the beaker, place the correctly labeled 200- X 25-mm culture tube. Tare these containers.

(17) With a Teflon-coated spatula, carefully transfer the tissue homogenate to the tared culture tube.

(18) With a Kimwipe, wipe the top and outside of the culture tube's lip.

(19) Record the weight.

(20) Seal the tube with a piece of Parafilm and place the tube in a rack.

(21) To each of the culture tubes with the weighed homogenates, add (a) an equal weight of 6 M hydrochloric acid (i.e., more easily measured as 1 ml acid/1 g tissue), and (b) 25 ml of methylene chloride.

(22) Cross two pre-cut pieces of Teflon tape over the mouth of each tube before capping tightly with a lid and shake each tube vigorously by hand for 30 s.

(23) Securely place all culture tubes on the Rototorque and set the controller to ~6.5 on high speed.

(24) After 1 hr, move the tubes from the Rototorque to a tube rack.

(25) For each sample, vigorously shake the culture tube and evenly divide the slurry between the two corresponding 127- X 28-mm round-bottom centrifuge tubes. (A two-pan balance can be used to verify even-weightedness.)

(26) To each emptied culture tube, add 25 ml of fresh methylene chloride, recap each tube with the Teflon-tape-lined lid, and shake the tubes for ~15 s.

(27) Cover the mouths of the centrifuge tubes with Parafilm and place the tubes in the centrifuge.

(28) Set the centrifuge at 3000 rpm for 5 min (start at speed setting 3.5 for ~1 min, then set the speed to 5 for the remaining time).

(29) When the centrifuge has completely stopped, move the tubes to the tube rack, pairing tubes with their partners.

(30) Transfer the tape labels from the empty polycarbonate centrifuge tubes to 125-ml Erlenmeyer flasks.

(31) With a 5- to 10-ml pipettor, transfer the bottom (methylene chloride) layers of the first two matched centrifuge tubes to the appropriately labeled Erlenmeyer flask and place the used pipet tip in the flask.

(32) Put a fresh tip on the pipettor and proceed to transfer the remaining extracts in a similar manner, using a fresh pipet tip for each sample and being sure to transfer both fractions of each sample to the appropriate flask.

(33) From each culture tube, evenly divide the methylene chloride rinse between the two corresponding centrifuge tubes (which contain the 1X extracted HCl/tissue mixture).

(34) Cover the mouth of each centrifuge tube with a piece of Teflon tape.

(35) With a polyethylene glove on your hand, securely stopper the taped mouth with your thumb and vigorously shake each tube for 30 s. (Change the glove when it becomes soiled.)

(36) Place the centrifuge tubes in the centrifuge and centrifuge the samples as in step 28.

(37) Using the 5- to 10-ml pipettor and the tips from the Erlenmeyer flasks, carefully transfer the corresponding methylene chloride extracts as described in step 31. Be sure not to cross-contaminate samples by mixing up tips. (If this is a concern, always use fresh tips.) Discard all soiled tips.

(38) Place the Erlenmeyer flasks in an approximately 5- X 30- X 19-cm tray (six flasks per tray) and fill the tray halfway with warm (~45°C) tap water.

(39) Into each Erlenmeyer flask's mouth, position a mini-vap outlet and set the argon or nitrogen gas pressure for a slow, steady stream.

(40) Every 15-20 min, carefully scoop out and discard the cooled water from the pan, being sure not to tip the flasks, and refill the pan with warm tap water.

(41) When each extract reaches dryness, secure a square of Parafilm over the mouth of each Erlenmeyer flask.

(42) Store the flasks in the refrigerator overnight.

3.2 TISSUE EXTRACT RECONSTITUTION, DAY 2

Equipment

Glassware

20-ml borosilicate scintillation vials with polyethylene-lined caps, one per sample

Chemical Solvents

Toluene ($C_6H_5CH_3$), ~5 ml per sample

Other Materials

1000-μl pipettor
Disposable tips for 1000-μl pipettor

Procedure

(1) Bring the refrigerated Erlenmeyer flasks that contain the dried extracts to room temperature.

(2) Label 20-ml borosilicate scintillation vials, one per sample.

(3) With a 1000-μl pipettor, add 3 ml toluene to each flask. (If you have an expectation that the TBT concentration will be >2 μg/g wet tissue, add 5 ml toluene.)

(4) Swirl and roll each flask so that the toluene rinses the entire inner surface of the flask, being careful not to spill any of the rinse, and transfer the reconstituted extract to the appropriate scintillation vial.

This extract can now be prepared for GF-AAS and/or GC analysis as described in sections 3.3 and 3.4, respectively.

3.3 EXTRACT PREPARATION FOR GF-AAS ANALYSIS

Equipment

Graphite Furnace-Atomic Absorption Spectrophotometer
(see section 4.1 for associated equipment and setup parameters)

Glassware and Plasticware

16- X 100-mm PYREX culture tubes, two per sample
Polypropylene screw caps for culture tubes
Small, ~6.5 cm top diameter, disposable polystyrene funnels, one per sample

Chemical Solvents and Reagents

3-percent aqueous sodium hydroxide solution (NaOH in distilled water) stored over a layer of methylene chloride (CH_2Cl_2), ~2 ml per sample

Sodium chloride (NaCl), ~0.5 g per sample

Standard of tributyltin chloride [$(C_4H_9)_3SnCl$] in toluene, ~1-1.5 μg/ml

Toluene ($C_6H_5CH_3$), ~5 ml per sample

Isopropyl alcohol [$(CH_3)_2CHOH$], ~5 ml per sample

0.1 M ammonium dichromate [$(NH_4)_2Cr_2O_7$], ~1 ml per sample

Other Materials

Polyethylene gloves

Rack for 16- X 100-mm culture tubes

Teflon-coated spatula

5-, 10-, 20-, 50-, 100-, 500-, and 1000-μl pipettors and tips

Vortex mixer (VWR)

Polyethylene AA cups, ~5-10 per sample

AA cup rack

Procedure for GF-AAS Analysis of TBT*

(1) To prepare the primary tributyltin chloride (TBTCl) standard (1° STD), begin by weighing (to 0.0001 g) a 100-ml volumetric flask.

(2) Add a portion of a drop of TBTCl from a 10-μl pipettor tip to the flask.

(3) Reweigh the flask.

(4) Carefully add toluene to bring the standard to the 100-ml volume mark, cap the flask, and shake it well (polyethylene gloves should be worn).

(5) Calculate the concentration (μg/ml) of TBTCl in the volumetric flask.[1]

(6) If the 1° STD is more concentrated than ~1.5 μg TBTCl/ml, a secondary standard (2° STD) must be made.[2] Shake the 1° STD, and with a pipettor(s) transfer an appropriate amount of 1° STD to a clean volumetric flask. Bring this 2° STD to volume by carefully adding toluene, then cap and shake the flask.

(7) Label 16- X 100-mm PYREX culture tubes (two per sample plus two for the TBTCl standard, putting one set aside) and place them in a rack.

(8) To the first set of culture tubes, add ~0.5 g (two scoops with a Teflon-coated spatula) sodium chloride through a disposable funnel.

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*Superscript number after a procedural step refers to corresponding step number in the sample calculation, section 5.

(9) With a 1000-µl pipettor, add 2 ml of 3-percent sodium hydroxide solution to each tube intended for the samples, and 3-4 ml to the tube for the STD, being careful not to pick up any of the methylene chloride layer beneath the sodium hydroxide.

(10) Into each sample in the scintillation vials, pump a 1000-µl pipettor to mix the extract, transfer 2 ml of extract to the appropriate culture tube, cap the tube tightly, and place the tube on a vortex mixer for 30 s. Be sure to use a fresh pipet tip for each sample, and try not to touch the tip to the interior of the culture tube between transfers.

(11) For the TBTCl STD (~1-1.5 µg TBTCl/ml), shake the STD and pipet 3-4 ml to its labeled culture tube, cap, and mix as described in step 10.

(12) After the two layers in each tube separate completely (5 min or more; some tubes may require light tapping to release bubbles of the organic layer from the salt), pipet most of the top layer to the empty corresponding tube of the second set. Again, be sure to use a fresh pipet tip for each sample. This aliquot of extract will be analyzed by GF-AAS.

Before analyzing the tissue extracts, a standard curve (absorbance vs. concentration) should be produced of the sodium hydroxide-treated TBTCl STD to assess the working status of the GF-AAS. To do this:

(13) Set up five AA cups within the AA cup rack.

(14) Add the TBTCl standard and matrix modifiers by following the outline below, using the appropriate pipettors and being sure to change tips after each use to avoid contamination.

Cup No.	µl of STD	µl of Toluene	µl of Isopropyl Alcohol	µl of 0.1 M Ammonium Dichromate
0	0	300	600	100
1	5	300	600	100
2	10	300	600	100
3	25	300	600	100
4	50	300	600	100

(15) With a 1000-µl pipettor, mix the contents of each cup by repeatedly (three to five times) pumping the pipettor in the solution. Use a clean tip in each cup.

(16) Place cup 0 in the AZ slot and cups 1-4 in slots 1-4 of the AA's autosampler tray.

(17) Autozero the AA on cup 0.

(18) Analyze the samples.

(19) Record the absorbance units and plot these against their calculated concentrations (μg TBTCl/ml in AA cup).[3]

(20) If a straight line intersecting 0,0 is not produced, adjust the GF-AAS as necessary and repeat steps 13-19.

(21) Now set one AA cup per sample into the rack and pipet the following into each cup, being sure to change tips when appropriate:

- 100 μl extract,
- 200 μl toluene,
- 600 μl isopropyl alcohol, and
- 100 μl 0.1 M ammonium dichromate.

(22) Mix the contents of each cup as described in step 15.

(23) Place the sample cups into the autosampler tray and note their positions.

(24) Analyze the samples, and record the absorbance units.

(25) If any of the extracts give absorbance units in excess of 0.025, these samples will need to be diluted so that they will give readings of ~0.020 (i.e., values between 0.010 and 0.025).[4]

(26) After the dilution factor to obtain ~0.020 absorbance has been determined, prepare five cups for the first sample as follows (Note: preparing more than five cups at one time leads to precipitation before the samples are analyzed):

Cup No.	μl of Extract	μl of STD*	μl of Toluene	μl of Isopropyl Alcohol	μl of 0.1 M Ammonium Dichromate
1	0	0	300	600	100
2	X	0	300	600	100
3	X	5	300	600	100
4	X	10	300	600	100
5	X	25	300	600	100

*the sodium hydroxide-treated TBTCl STD (1-1.5 μg TBTCl/ml).

X = the amount (diluted or not) of sample needed to give an absorbance between 0.010 and 0.025 with no standard added, i.e., cup 2.

Use fresh pipet tips as necessary and be sure to thoroughly mix the solution in each cup as described in step 15.

(27) Place cup 0, the blank, in the AZ slot for autozeroing and the remaining cups in consecutive slots, noting their positions.

(28) Analyze the samples.

(29) Record the absorbances, and plot absorbance vs. concentration of TBTCl standard in the cups.[3]

(30) For any point that does not fall on the line, prepare a fresh cup of extract, standard, and matrix modifier to generate a new point at this concentration.

(31) Run a linear regression of the data and calculate the amount of tin in the tissue sample (μg Sn/g wet tissue).[5]

To obtain a more accurate tin content of the samples (optional):

(32) Follow step 26, using half as much tissue extract.

(33) Plot the data as described in step 29.

(34) Run the linear regression and again calculate the amount of tin in the tissue sample (μg Sn/g wet tissue).

(35) For the two dilutions of extracts analyzed here and in step 26, plot μg Sn/g wet tissue vs. percent dilution of the extract.[6]

(36) Run a linear regression and find the concentration of tin in the tissue at infinite dilution (i.e., x = 0, the y intercept). This should be a better representation of the tin content if there were no matrix effects.

3.4 EXTRACT PREPARATION FOR GC-FID AND GC-MS ANALYSIS

Equipment

Gas Chromatograph (see sections 4.2 and 4.3 for associated equipment and setup parameters)

Glassware

16- X 100-mm PYREX culture tubes, one or three* per sample
Polypropylene screw caps for culture tubes
1-ml crimp-seal vials with Teflon-lined septum seals, one per sample
Miniature champagne columns, one per sample*

Chemical Solvents and Reagents

Internal Standard of dipropyltin dichloride ($[C_3H_7]_2SnCl_2$) in toluene (3-4 mg/ml), 10 μl per sample
2 M hexylmagnesium bromide ($C_6H_{13}MgBr$) in ether (AGFA VENTRON), 100 μl per sample
1 N sulfuric acid (H_2SO_4), 2 ml per sample
Sodium chloride (NaCl), ~0.5 g per sample
Hexane (C_6H_{14}), 3 ml per sample*

*Materials needed for the optional Florisil cleanup procedure. See note below step 6.

Other Materials

Labeling tape, ~1 in. per sample
100-, 500-, and 1000-µl pipettors with disposable tips
Crimper for crimp-seal vials
Silanized glass wool, ~1 cm^2 per sample*
Florisil, ~1 ml per sample*
Compressed argon or nitrogen
Mini-vap (SUPELCO), one outlet per sample

Procedure for GC Analysis of TBT and DBT

(1) Mark 16- X 100-mm culture tubes (one per sample) and place them in a rack.

(2) Prepare no more than six derivatizations (samples) at a time. Using pipettors and changing pipet tips after each use, add the following to the culture tubes:

(a) 10 µl internal standard (dipropyltin dichloride)
(b) 500 µl of the corresponding, well-mixed extract from the scintillation vial
(c) 100 µl of 2 M hexylmagnesium bromide in ether

Cap the tube immediately after step (c) and mix the solution on a vortex mixer for 30 s, rolling the tube to rinse the inner walls.

(3) Let the solution react for 20 min. In the meantime, prepare the next five to six samples.

(4) After the 20-min reaction time, hydrolyze the sample by pipetting 2 ml 1 N sulfuric acid into each tube, capping the tube immediately and placing the tube on the vortex mixer for 30 s.

(5) Using one disposable funnel per sample, add ~0.5 g (two scoops with a Teflon spatula) sodium chloride to each derivatized extract.

(6) Recap and mix the tube on a vortex mixer for 30 s.

If the samples are to be analyzed by GC-FID, proceed with step 11. If the samples will be analyzed by GC-MS, either continue at step 7 for immediate analysis or proceed to step 11 for Florisil cleanup.

(7) Label 1-ml crimp-seal vials (one per sample) with labeling tape.

(8) After each derivatized sample has separated from the aqueous layer (>5 min), carefully transfer the top layer to the corresponding vial. Be sure to use a fresh pipet tip for each sample and be careful not to transfer any of the bottom layer to the vial.

(9) Cap the vials, using the crimper.

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*Materials needed for the optional Florisil cleanup procedure. See note below step 6.

(10) Analyze the derivatized extracts by GC-MS and calculate the amount of TBT and DBT as tin in the tissue (μg Sn/g wet tissue).[7]

If the extracts will be cleaned with Florisil, proceed with the following steps (11-25).

(11) Using forceps and a long-nosed Pasteur pipet, pack a small plug of silanized glass wool into the tip of each champagne column (one column per sample).

(12) Carefully fill the stem of the column with Florisil, lightly tapping the column as it fills. The level of Florisil should just reach the base of the cup.

(13) Set each column in a 16- X 100-mm culture tube standing in a rack.

(14) Pipet 2 ml of hexane onto the top of the packing, letting the first 1 ml partially drain before adding the remaining 1 ml.

(15) Discard the hexane and culture tubes after the champagne columns have drained.

(16) Label 16- X 100-mm culture tubes (one per sample) and place one under each champagne column.

(17) Using a 100-μl pipettor and a fresh tip for each sample, apply 300 μl of the derivatized extract (top layer) from step 8 to the corresponding champagne column.

(18) After each extract drains into the Florisil, carefully pipet 3 ml of hexane into each cup (1 ml at a time, waiting for the previous rinse to just reach the top of the Florisil before adding the next aliquot).

(19) Let the hexane completely drain from the Florisil.

(20) Place each of the culture tubes with the collected eluents under an outlet of the mini-vap, and set the argon or nitrogen regulator for a slow, steady stream.

(21) When the samples reach dryness, pipet 300 μl toluene into each tube and swirl and roll the tubes to rinse the dried extracts from the inner walls.

(22) Label 1-ml crimp-seal vials (one per sample) with labeling tape.

(23) With a fresh tip for each sample, pipet the samples from the culture tubes to their corresponding vials.

(24) Crimp-seal the vials and analyze the samples by GC-FID.

(25) Calculate the amount of TBT and DBT as tin in the tissue (μg Sn/g wet tissue).[7]

Note: For GC analyses, be sure that the instruments are calibrated to known TBT and DBT standards and to the particular batch of internal standard which is being added to the samples.

4. SUMMARY OF INSTRUMENTATION

4.1 GF-AAS

Equipment

Perkin-Elmer 5000 Atomic Absorption Spectrophotometer
Perkin-Elmer 8-W Electrodeless Tin Discharge Lamp
Perkin-Elmer EDL Power Supply
Perkin-Elmer AS40
Perkin-Elmer HGA Programmer
Perkin-Elmer Atomic Spectroscopy Data System 10
Perkin-Elmer PR-100 Printer
Perkin-Elmer 056 Strip Chart Recorder
Perkin-Elmer graphite tube with platform
UPC compressed argon
Cooling water - house supply

Parameters

Perkin-Elmer 5000 AAS
time: 8.0 s
Energy: 60
slit Low: 0.7 nm
wavelength: 224.6 nm
Record Absorbance: 1
Average: 3
CV: 1
Atomic Absorption-Background Corrector (AA-BG): ON
Absorbance: ON
Peak Height: ON

Perkin-Elmer AS40

25-μl sample volume

Perkin-Elmer HGA 500

Step	1	2	3	4
Temperature	100	1000	2700	20
Ramp Time	20	10	0	10
Hold Time	20	0	7	10
Recorder on			-4	
Read			-2	
Base Line			-1	
Internal Flow			0	

Gas

Argon, 50 psi

4.2 GC-FID

Equipment

Hewlett-Packard 5880A Series Gas Chromatograph with Flame Ionization Detector
Hewlett-Packard GC Terminal
Supelco SPB-1 Fused-Silica Capillary Column, 30 m X 0.25 mm ID with 0.25 μm film thickness
UPC Compressed helium
Compressed hydrogen
Compressed air

Parameters

Oven Temperature Profile and Heated Zones:

Initial Temperature: 50°C
Initial Time: 2.00 min
Program Rate: 30.00°C/min
Final Value: 200°C
Final Time: 7.50 min
Post Value: 280°C
Post Time: 20.00 min
Detector Temperature: 300°C
Injector Temperature: 250°C
Purge Off Time: 0.60 min

Gas

Helium, 30 psi
Hydrogen, 45 psi
Argon, 35 psi

Splitless Injection

Sample Size: 1 μl

Identifying Retention Times

Tributylhexyltin: 8.28 min
Dipropyldihexyltin (internal standard): 8.49 min
Dibutyldihexyltin: 9.83 min

4.3 GC-MS

Equipment

Hewlett-Packard 5890 Gas Chromatograph
Hewlett-Packard 5970 Series Mass Selective Detector with direct capillary inlet
Hewlett-Packard 59970 MS Chemstation

HP-1 Fused-Silica Column, 12 m X 0.2 mm with 0.33 μm film thickness
UPC Compressed helium

Parameters

Oven Temperature Profile and Heated Zones:

Initial Temperature: 50°C
Initial Time: 3.00 min
Program Rate: 30.00°C/min
Final Temperature: 200°C
Final Time: 6.5 min
Standby Temperature: 225°C
Manual Delays Between Runs: 15 min
Injector Temperature: 250°C
Transfer Line Temperature: 250°C
Purge Off Time: 0.60 min

Selective Ion Mode Aquisition

Electron Multplier Voltage: 1800 V
m/z: 85, 119, 121, 177, and 191
Dwell: 50
Cycles per second: 3.1

Pressure Settings

Column Head Pressure: 5 psi
Source Pressure: 5 X 10^{-5} torr

Splitless Injection

Sample Size: 1 μl

Indentifying Retention Times

Tributylhexyltin: 8.28 min
Dipropyldihexyltin: 8.49 min
Dibutyldihexyltin: 9.83 min

5. SAMPLE CALCULATIONS

Step No.

1 Concentration of prepared TBTCl STD (μg/ml) = A

B weight of empty flask (g)
C weight of flask with TBTCl added (g)
D volume of toluene added to flask (ml)

$$\frac{C - B}{D} \times 10^6 = A$$

2 Dilution of 1° STD to yield 2° STD of ~1-1.5 μg TBTCl/ml:

A concentration of 1° STD (μg TBTCl/ml)
E desired final concentration of 2° STD (1-1.5 μg TBTCl/ml)
F final prepared volume of 2° STD (ml)
G volume of 1° STD to be diluted to F in volumetric flask (ml)

$$\frac{E \times F}{A} = G$$

3 Concentration of TBTCl STD in AA cup (μg/ml) = H

I concentration of sodium hydroxide-treated STD (μg TBTCl/ml)
J amount of STD added to AA cup (μl)
K total volume in AA cup (μl)

$$\frac{I \times J}{K} = H$$

4 Dilution of extracts to obtain absorbances of ~0.020:

L amount of extract initially analyzed (μl)
M absorbance reading obtained from L
N dilution factor to obtain an absorbance of ~0.020

$$\frac{M}{0.02} = N$$

either (a) dilute a portion of the original extract to 1/N its concentration and analyze as before, or (b) prepare an AA cup as before, but add 1/N of L.

For example:

100 μl of extract gave an absorbance reading of 0.042, but an absorbance of ~0.020 is wanted. Thus

L = 100 μl
M = 0.042

and from above:

$$N = \frac{0.042}{0.02} \sim 2 \quad .$$

Therefore either (a) dilute some of the extract to one-half its concentration with toluene in a clean 16- X 100-mm culture tube and analyze 100 µl of the diluted extract as described in step 21, section 3.3, or (b) analyze one-half as much of the original extract by preparing the sample as in step 21, except use 50 µl of extract instead of 100 µl.

Whichever method of dilution is chosen, be sure that the AA cup solution ratio is ~3:6:1 of toluene, isopropyl alcohol, and 0.1 M ammonium dichromate, respectively.

5 Concentration of tin extracted as TBT from the tissue (µg Sn/g wet tissue), as determined by GF-AAS = P

From the linear regression of absorbance vs. concentration of TBTCl STD (µg TBTCl/ml) in the AA cups, solve the equation for y = 0. This is (-) the concentration of TBT in the cup which contains the extract but no added standard. Call this value Q.

- N dilution factor calculated from step 4, if there is one
- R amount of extract added to AA cup (µl)
- S total volume in AA cup (µl)
- T concentration of TBTCl in the extract (µg/ml), calculated below
- U amount of toluene used to reconstitute dried extract (ml)
- V wet weight of tissue extracted (g)
- W concentration of TBT extracted from the tissue (µg TBTCl/g wet tissue), calculated below
- Y fraction of TBTCl as tin, 0.37

$$\frac{S \times Q}{R} = T$$

$$\frac{T \times N \times U}{V} = W$$

$$W \times Y = P$$

6 Percent dilution of the extract = Z

- R amount of extract added to AA cup (µl)
- S total volume in AA cup (µl)
- N dilution factor calculated from step 4, if there is one

$$\frac{R}{N \times S} \times 100 = Z$$

7 Concentration of tin extracted as TBT and DBT from tissue (µg Sn/g wet tissue), as determined by GC:

- U amount of toluene used to reconstitute dried extract (ml)

V wet weight of tissue extracted (g)
Y fraction of TBTCl as tin, 0.37
EE fraction of DBTCl as tin, 0.40
AA concentration of TBT in derivatized extract analyzed by GC (μg TBTCl/ml)
BB concentration of DBT in derivatized extract analyzed by GC (μg DBTCl/ml)
CC total volume in vial (μl)
DD amount of extract in vial (μl)
FF concentration of tin extracted from tissue as TBT (μg Sn/g wet tissue)
GG concentration of tin extracted from tissue as DBT (μg Sn/g wet tissue)

$$\frac{AA \times CC \times U \times Y}{DD \times V} = FF$$

$$\frac{BB \times CC \times U \times EE}{DD \times V} = GG$$

6. CONCLUDING REMARKS

Tissue of marine organisms can be effectively analyzed by GF-AAS, GC-FID, or GC-MS for organotins as described. However, experience at NOSC with these procedures gives rise to the following observations and theories:

(1) Tin values obtained by GF-AAS may be artificially low or high due to unknown compounds in the tissue extracts that act as Sn signal suppressors or enhancers.

(2) There is a possibility for breakdown of TBT in the extracts prepared for GF-AAS analysis. In a simple solvent system, different butyltin species give different AAS responses per μg Sn analyzed. However, by adding the ammonium dichromate matrix modifier, the AAS response to Sn is better smoothed for the varying butyltin species. Thus, breakdown products should cause a minimal problem when quantifying Sn content of tissue extracts. In addition, the ammonium dichromate enhances the GF-AAS signal to Sn so that samples with low levels of Sn are more easily quantified.

(3) Some alkylmagnesium bromide reagents can be contaminated with TBT and/or DBT. This contamination can lead to significant introduction of variable amounts of TBT and DBT into the samples, thus causing quantification problems, especially in samples containing low levels of these compounds. It is therefore recommended that before using a particular batch of derivatizing agent on valuable samples, it be checked for purity by running blanks of the stock.

Work will continue to correct for these analytical inefficiencies.

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